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(54) Title: USE OF AGENTS WHICH BLOCK INTERCELLULAR ADHESION MOLECULE/RECEPTOR INTERACTION IN THE TREATMENT OF RESPIRATORY VIRAL INFECTION

(57) Abstract

The present invention relates to the use of intercellular adhesion molecules (ICAM-1), their functional derivatives, and molecules which bind to them, in methods to increase gas exchange in the lungs of a patient suffering from a respiratory viral infection.

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Use of Agents Which Block Intercellular Adhesion Molecule/Receptor Interaction in the Treatment of Respiratory Viral Infection

Background of the Invention

Field of the Invention

The present invention is directed to the use of agents which block ICAM-1/receptor interactions as a means to increase gas exchange in the lungs of a patient suffering from a viral infection of the respiratory tract.

Description of the Related Art

I. Leukocyte Adhesion

Leukocytes must be able to attach to cellular substrates in order to properly defend the host against foreign invaders such as bacteria or viruses. An excellent review of the defense system is provided by Eisen, H.W., (In: *Microbiology*, 3rd Ed., Harper & Row, Philadelphia, PA (1980), pp. 290-295 and 381-418). Leukocytes attach to endothelial cells so that they can migrate from circulation to sites of ongoing inflammation. Furthermore, leukocytes attach to antigen-presenting cells so that a normal specific immune response can occur. Finally, leukocytes attach to appropriate target cells so that lysis of virally-infected or tumor cells can occur.

II. CD18 Family

Leukocyte surface molecules involved in mediating such attachments have been identified using hybridoma technology. Briefly, monoclonal antibodies ("MAbs") directed against human T-cells (Davignon, D. *et al.*, *Proc. Natl. Acad. Sci. USA 78*:4535-4539 (1981)) and mouse spleen cells (Springer, T. *et al. Eur. J. Immunol. 9*:301-306 (1979)) were identified which

bound to leukocyte surfaces and inhibited the attachment related functions described above (Springer, T. *et al., Fed. Proc. 44*:2660-2663 (1985)). The molecules identified by these antibodies are called Mac-1, p150,95 and Lymphocyte Function-associated Antigen-1 (LFA-1). Mac-1 is found on macrophages, granulocytes and large granular lymphocytes. LFA-1 is found on most lymphocytes (Springer, T.A., *et al. Immunol. Rev. 68*:111-135 (1982)). These two molecules, plus p150,95 (which has a tissue distribution similar to Mac-1) play a role in cellular adhesion (Keizer, G. *et al., Eur. J. Immunol. 15*:1142-1147 (1985)). Molecules such as these three members of the LFA-1 family, which are involved in the process of cellular adhesion, are referred to as "adhesion molecules."

The above-described leukocyte molecules were found to be structurally similar to one another, and to constitute members of a related family of glycoproteins (Sanchez-Madrid, F. *et al., J. Exper. Med. 158*:1785-1803 (1983); Keizer, G.D. *et al., Eur. J. Immunol. 15*:1142-1147 (1985)). This glycoprotein family is composed of heterodimers having one alpha subunit and one beta subunit. Although the alpha subunit of each of the antigens differs from one member to the next, the beta subunit of each member is highly conserved (Sanchez-Madrid, F. *et al., J. Exper. Med. 158*:1785-1803 (1983)). The beta subunit of the glycoprotein family (referred to as "CD18" family) was found to have a molecular weight of 95 kd whereas the alpha subunits were found to vary from 150 kd to 180 kd (Springer, T., *Fed. Proc. 44*:2660-2663 (1985)). Although the alpha subunits of the membrane proteins do not share the extensive homology shared by the beta subunits, close analysis of the alpha subunits of the glycoproteins has revealed that there are substantial similarities between them. Reviews of the similarities between the alpha and beta subunits of the LFA-1 related glycoproteins are provided by Sanchez-Madrid, F. *et al.* (*J. Exper. Med. 158*:586-602 (1983); *J. Exper. Med. 158*:1785-1803 (1983)).

Individuals have been identified who are unable to express normal amounts of any member of this adhesion protein family on their leukocyte cell

surfaces (Anderson, D.C., *et al., Fed. Proc. 44*:2671-2677 (1985); Anderson, D.C., *et al., J. Infect. Dis. 152*:668-689 (1985)). The condition is known as "Leukocyte Adhesion Deficiency" or "LAD" syndrome. Leukocytes from these patients displayed *in vitro* defects similar to normal counterparts whose CD18 family of molecules had been antagonized by antibodies. Furthermore, these individuals are unable to mount a normal immune response due to an inability of their cells to adhere to cellular substrates (Anderson, D.C., *et al., Fed. Proc. 44*:2671-2677 (1985); Anderson, D.C., *et al., J. Infect. Dis. 152*:668-689 (1985)). LAD individuals present clinically with delayed umbilical cord separation, recurring and progressive soft tissue infections, and impaired pus formation, despite a striking blood leukocytosis. Studies of LAD individuals have revealed that immune reactions are mitigated when leukocytes are unable to adhere in a normal fashion due to the lack of functional adhesion molecules of the CD18 family.

III. ICAM-1

ICAM-1 is a single chain glycoprotein varying in mass on different cell types from 76-114 kD, and is a member of the Ig superfamily with five C-like domains (Dustin, M.L. *et al., Immunol. Today 9*:213-215 (1988); Staunton, D.E. *et al., Cell 52*:925-933 (1988); Simmons, D. *et al., Nature 331*:624-627 (1988)). ICAM-1 is highly inducible with cytokines (including IFN-γ, TNF, and IL-1) in a wide range of cell types (Dustin, M.L. *et al., Immunol. Today 9*:213-215 (1988)). Induction of ICAM-1 on epithelial cells, endothelial cells, and fibroblasts mediates LFA-1 dependent adhesion of lymphocytes (Dustin, M.L. *et al., J. Immunol. 137*:245-254 (1986); Dustin, M.L. *et al., J. Cell. Biol. 107*:321-331 (1988); Dustin, M.L. *et al., J. Exp. Med. 167*:1323-1340 (1988)). Adhesion is blocked by pretreatment of lymphocytes with LFA-1 MAb or pretreatment of the other cell with MAb to ICAM-1 (Dustin, M.L. *et al., J. Immunol. 137*:245-254 (1986); Dustin, M.L. *et al., J. Ce Biol. 107*:321-331 (1988); Dustin, M.L. *et al., J. Exp. Med. 167*:1323-1340

(1988)). Identical results with purified ICAM-1 in artificial membranes or on Petri dishes demonstrate that LFA-1 and ICAM-1 are receptors for one another (Marlin, S.D. *et al., Cell 51*:813-819 (1987); Makgoba, M.W. *et al., Nature 331*:86-88 (1988)). For clarity, LFA-1 and ICAM-1 are referred to herein as "receptor" and "ligand," respectively. Further descriptions of ICAM-1 are provided in U.S. Patent Applications Serial Nos. 07/045,963; 07/115,798; 07/155,943; 07/189,815 and 07/250,446, all of which applications are herein incorporated by reference in their entirety.

IV. Respiratory Viral Infection

The ability of leukocytes, especially lymphocytes to maintain the health and viability of an animal requires that they be capable of adhering to other cells (such as endothelial cells). This adherence has been found to require cell-cell contacts which involve specific receptor molecules present on the cell surface of the lymphocytes. These receptors enable a lymphocyte to adhere to other lymphocytes or to endothelial, and other non-vascular cells. The cell surface receptor molecules have been found to be highly related to one another. Humans whose lymphocytes lack these cell surface receptor molecules exhibit defective antibody responses, chronic and recurring infections, as well as other clinical symptoms.

Acute viral respiratory illnesses are among the most common of human diseases, accounting for one-half or more of all acute illnesses. The incidence of acute respiratory disease in the United States is from 3 to 5.6 cases per person per year. The highest rates occur in children under 1 year of age (6.1 to 8.3 cases per year) and remain high until age 6, when a progressive decrease is noted. Adults in the general population have three to four illnesses per person per year. Morbidity from acute respiratory illnesses accounts for 30 to 50 percent of time lost from work by adults and from 60 to 80 percent of time lost from school by children.

It has been estimated that two-thirds to three-fourths of cases of acute respiratory illnesses are caused by viruses. More than 200 antigenically distinct viruses from 8 different genera have been reported to cause acute respiratory illness, and it is likely that additional agents will be described in the future. The vast majority of these viral infections involve the upper respiratory tract, but lower respiratory tract disease can also occur, particularly in younger age groups and in certain epidemiologic settings.

The illnesses caused by respiratory viruses traditionally have been divided into multiple distinct syndromes, such as the "common cold", pharyngitis, croup (laryngotracheobronchitis) tracheitis, bronchiolitis, bronchitis, and pneumonia. These general categories of illnesses have a certain epidemiologic and clinical utility, e.g., croup occurs exclusively in very young children and has a characteristic clinical course. In addition, some types of respiratory illnesses are more likely to be associated with certain viruses, e.g., the "common cold" with rhinoviruses, while others occupy characteristic epidemiologic niches, such as adenoviruses in military recruits. The syndromes most commonly associated with infection with the major respiratory virus groups are summarized in Table 1. Despite these associations, it is clear that most respiratory viruses have the potential to cause more than one type of respiratory illness, and frequently features of several types of illness may be present in the same patient. Moreover, the clinical illnesses induced by these viruses are rarely sufficiently distinctive to enable an etiologic diagnosis to be made on clinical grounds alone, although the epidemiologic setting increases the likelihood that one group of viruses rather than another may be involved. In general, laboratory methods must be relied upon to establish a specific viral diagnosis.

Table 1 Illnesses Associated with Respiratory Viruses

Frequency of respiratory syndromes associated with virus groups

Virus	Most frequent	Occasional	Infrequent
Rhinoviruses	Common cold	Exacerbation of chronic bronchitis and asthma	Pneumonia (children)
Coronaviruses	Common cold	Exacerbation of chronic bronchitis and asthma	Pneumonia and bronchiolitis
Respiratory syncytial virus	Pneumonia and bronchiolitis in young children	Common cold in adults	Pneumonia in elderly
Parainfluenza viruses	Croup and lower respiratory tract disease in young children	Pharyngitis and common cold	Tracheobronchitis in adults
Adenoviruses	Common cold and pharyngitis in children	Outbreaks of acute respiratory disease (ARD)* in military recruits	Pneumonia in children and immunosuppressed patients
Influenza A viruses	"Influenza-like illness"†	Pneumonia and excess mortality in "high-risk" patients	Pneumonia in healthy individuals
Influenza B viruses	"Influenza-like illness"†	Rhinitis and pharyngitis alone	Pneumonia
Enteroviruses	Acute undifferentiated febrile illnesses‡	Rhinitis and pharyngitis	Pneumonia
Herpes simplex viruses	Gingivostomatitis‡ (children); pharyngotonsillitis (adults)	Tracheitis and pneumonia in immunocompromised patients	Disseminated infection in immunocompromised patients

** Serotypes 4 and 7.* *† Fever, cough, myalgia, malaise.* *‡ May or may not have a respiratory component.*

Summary of the Invention

The present invention is based on the observation that agents which block ICAM-1/receptor interactions increase the rate of gas exchange in the lungs of a mammal which is suffering from a reduction in gas exchange as a result of a viral infection of the respiratory tract. Surprisingly, these agents do not affect the airway hyperresponsiveness (e.g., exacerbation of asthma) which also occurs as a result of the viral infection.

Based on these observations, the present invention provides methods for increasing the rate of oxygen absorption and CO_2 elimination in the lungs of a mammal suffering from a viral infection of the respiratory tract. Specifically, the rate at which oxygen is absorbed into, and CO_2 eliminated from, the blood in the lungs of a mammal suffering from an infection of the respiratory tract can be increased by providing a therapeutically effective amount of an agent which is capable of blocking ICAM-1/receptor interactions.

Examples of the types of viral pathogens for which the present method can be applied include, but are not limited to, members of the Paramyxoviradae family, preferably viruses which are members of the Pneumovirus or Paramyxovirus genus. Specific viral pathogens include the Respiratory Syncytial Virus and Parainfluenza virus.

The present methods utilize agents which can be divided into two groups based on the molecule the agent binds to (i.e., interacts with). Group I agents are agents which bind to (interact with) ICAM-1 and block the binding of ICAM-1 to a natural receptor of ICAM-1. Group II agents are agents which bind to a receptor of ICAM-1 and block the binding of the receptor to ICAM-1.

The agents of the present invention include small molecules, peptides, carbohydrates, proteins and antibodies. A preferred class of agents of the present invention are antibodies, or fragments thereof containing the antigen

binding site, which bind to ICAM-1 (Group I agents) or to one or more members of the CD18 family of glycoproteins (Group II agents).

Brief Description of the Figures

Figure 1 demonstrates the total lung leukocytes recovered by whole lung lavage from naive (normal) mice versus mice six days after inoculation with control media, respiratory syncytial virus (RSV), RSV and treated with control non-specific rat IgG (3 mg/kg, b.i.d.), or RSV and treated with the rat anti-mouse ICAM-1 monoclonal antibody YN1/1.7 (3 mg/kg, b.i.d.). Bars represent the mean + S.E. for 5 animals per group. Asterisk (*) signifies significant protection by YN1/1.7 (anti-ICAM-1) compared to RSV alone as well as RSV plus rat IgG treatment ($p < 0.05$ by Student's t-test).

Figure 2 demonstrates the lung diffusion capacity for carbon monoxide (D_{Lco}) from naive (normal) mice versus mice six days after inoculation with control media, RSV, RSV and treated with control non-specific rat IgG (3 mg/kg, b.i.d.). Bars represent the mean + S.E. for 4-6 animals per group. Asterisk (*) signifies significant protection by YN1/1.7 (anti-ICAM-1) compared to RSV alone as well as RSV plus rat IgG treatment ($p < 0.05$ by Student's t-test).

Figure 3 demonstrates the inhaled methacholine PC_{100} (airway responsiveness) for mice six days after inoculation with control media, RSV, RSV and treated with control non-specific rat IgG (3 mg/kg, b.i.d.), or RSV and treated with the rat anti-mouse ICAM-1 monoclonal antibody YN1/1.7 (3 mg/kg, b.i.d.). Bars represent the mean + S.E. for 8-9 animals per group. The RSV-induced decrease in the PC_{100} (increase in airway responsiveness) was not prevented by anti-ICAM-1 (YN1/1.7).

Detailed Description of the Preferred Embodiments

The present invention is based on the observation that agents which block ICAM-1/receptor interaction increase the rate of gas exchange in the lungs of a mammal suffering from a reduction in gas exchange as a result of a viral infection of the respiratory tract. Based on these observations, the present invention provides methods for increasing the rate at which oxygen is absorbed into and CO_2 eliminated from the blood in the lungs of a mammal suffering from a viral infection of the respiratory tract, primarily the lower respiratory tract. Specifically, the rate of oxygen absorption and CO_2 elimination can be increased in a mammal suffering from an infection of the respiratory tract by providing a therapeutically effective amount of an agent which blocks ICAM-1/receptor interactions.

As used herein, an increase in the rate of gas exchange is said to occur if the rate of exchange of gases across the lung membrane is increased. An increase in gas exchange can result in an increase in the rate or extent of oxygen absorption and/or result in an increase in the rate of or extent of carbon dioxide elimination. A skilled artisan can readily adapt known procedures to determine the rate and extent of gas exchange in a particular mammal in response to a particular treatment.

As used herein, "a viral infection of the respiratory tract" refers to any viral mediated infection of cells which make up and comprise the respiratory tract. Such cells include, but are not limited to epithelial cells, fibroblasts, alveolar macrophages, dendritic cells, and infiltrating leukocytes (for a description of the various cell types which make up the respiratory tract see Plopper *et al.*, Section I in *Comparative Biology of the Normal Lung*, Vol. 1, Parent, R.A., ed., CRC Press Inc., Boca Raton, FL (1992)).

The methods of the present invention are intended for use for viruses which infect cells of the respiratory tract and further lead to an increase or induction of ICAM-1 expression. Since the present methods are directed to ameliorating a symptom common to respiratory viral infection and are not

directed at treating the specific viral agent, the present methods can be used to augment the treatment of a wide variety of viral pathogens. Examples of such viruses include, but are not limited to, members of the Paramyxoviradae family, more specifically viruses which are members of the Pneumovirus or Paramyxovirus genus. Specific viruses which can be treated using the herein disclosed methods are the Respiratory Syncytial Virus and Parainfluenza virus (for a review of respiratory viruses see Dolin, "Common Viral Respiratory Infections" in *Harrison's Principles of Internal Medicine*, 11 edition, McGraw-Hill N.Y. (1987) and Table 1).

In addition to the family of viruses specifically described above, the methods disclosed herein are effective in increasing the rate of gas exchange in the lungs for all viruses which infect cells of the respiratory tract and cause an induction or an increase in ICAM-1 expression on the surfaces of cells of the respiratory tract (e.g., endothelial, epithelial, fibroblasts, alveolar macrophages, lymphocytes, dendritic cells, etc.). As used herein, a virus is said to induce or increase ICAM-1 expression when a cell produces a higher level of ICAM-1 as a result of the viral infection. A skilled artisan can use known methods to assay for ICAM-1 expression *in vivo* or *in vitro* to determine if a particular virus induces ICAM-1 expression (for example, see Wagner *et al.*, *Science 247*:456-459 (1990)). Such procedures include, but are not limited to, direct assays, methods which use nucleic acid probes or ICAM-1 specific antibodies to directly measure the level of ICAM-1 expression, and indirect assays, methods which detect the presence of cytokines known to induce ICAM-1 expression. For example, interferon gamma, interleukin-1, and tumor-necrosis factor, are cytokines which are known to induce ICAM-1 expression (Wagner *et al.*, *Science 247*:456-459 (1990); Pober *et al.*, *J. Immunol. 137*:1893-1896 (1986)).

As used herein, an agent is said to "block ICAM-1/receptor interactions" if the agent is capable of reducing the rate at which ICAM-1 binds to a receptor. There are two targets for the agents of the present invention. Group I agents are agents which bind to ICAM-1 and block the

binding of ICAM-1 to a natural receptor of ICAM-1. Group II agents are agents which bind to a member of the CD18 family of glycoproteins. Group II agents can be designed to bind to all members of the CD18 family of glycoproteins or can be designed to bind to a specific member of the CD18 family (Springer T.A., *Nature 346*:425-434 (1990)).

Assays have been developed to determine if an agent can block ICAM-1/receptor interactions (see Rothlein *et al., J. Immunol. 137*:1270-1274 (1986); Smith *et al., J. Clin. Invest. 82*:1746-1756 (1986)) for examples). In general, these procedures compare the level of ICAM-1/CD18 interactions in the presence and absence of the agent which is tested. The format of such assays varies and can include the use of isolated ICAM-1 and/or CD18 protein, cells which naturally express ICAM-1 or CD18, or cells which have been altered to express ICAM-1 or CD18. A skilled artisan can readily use these methods, or a combination thereof, to isolate agents for use in the methods herein described.

A preferred class of agents of the present invention are antibodies, or fragments thereof containing the antigen binding site, which bind to ICAM-1 (Group I agents) or to a member of the CD18 family of glycoproteins (Group II agents). ICAM-1 and the members of the CD18 family of molecules are immunogenic molecules. Thus, a skilled artisan can routinely obtain antibodies which bind to ICAM-1 or one or more members of the CD18 family of molecules. Group I agents include antibodies, and fragments thereof, which bind to ICAM-1. Group II agent include antibodies, and fragments thereof, which bind a member of the CD18 family of glycoproteins.

The generation of anti-ICAM-1 and anti-CD18 antibodies is well known in the art (Harlow *et al., Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY (19)). In general, the antibody agents of the present invention may be obtained by introducing either a purified protein, or cells which express the desired protein, into an appropriate animal, for example by intraperitoneal injection, etc. The serum of such an animal may be removed and used as a source of polyclonal

antibodies capable of binding these molecules. Alternatively, a skilled artisan can remove splenocytes from such animals, fuse these spleen cells with a myeloma cell line to form a hybridoma cell which secretes a monoclonal antibody which binds ICAM-1 or a member of the CD18 family of molecules. The hybridoma cells, obtained in the manner described above may be screened using known methods to identify desired hybridoma cells that secrete an antibody which binds to either ICAM-1 or to members of the CD18 family of molecules (either the alpha or beta subunit).

Both polyclonal and monoclonal antibodies may be used in the methods of the present invention. Of special interest to the present invention are antibodies to ICAM-1 or to members of the CD18 family, which are produced in humans, or are "humanized" (i.e., non-immunogenic in a human) by recombinant or other technology. Humanized antibodies may be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e., chimeric antibodies) (Better, M. *et al.*, *Science 240*:1041-1043 (1988); Liu, A.Y. *et al.*, *Proc. Natl. Acad. Sci. USA 84*:3439-3443 (1987)), or through the process of complement determination region (CDR) grafting (Jones, P.T. *et al.*, *Nature 321*:552-525 (1986); Verhoeyan *et al.*, *Science 239*:1534 (1988); Beidler, C.B. *et al.*, *J. Immunol. 141*:4053-4060 (1988)).

Another class of agent which can be used in the present invention are soluble forms of ICAM-1 or members of the CD18 family of glycoproteins. Because ICAM-1 binds to a CD18 molecule, soluble derivatives of ICAM-1 comprise another type of Group II agent. Soluble derivatives of ICAM-1 which bind to the CD18 family member reduce the rate of ICAM-1/receptor binding by competing with the CD18 found on leukocytic cells, thus attenuating cellular adhesion.

ICAM-1 is composed of 5 domains (Staunton, D.E. *et al.*, *Immunol. Today 9*:213-215 (1988); Staunton, D.E. *et al.*, *Cell 52*:925-934 (1988); Staunton, D.E. *et al.*, *Cell 56*:849-854 (1989); Staunton, D.E. *et al.*, *Tissue Antigens 33*:287 (1989)). Domains 1 and 2 have been found to be important

for the binding of ICAM-1 to its receptor molecule (Staunton, D.E. *et al., Tissue Antigens 33*:286 (1989); Staunton, D.E. *et al., FASEB J. 3*:A446 (1989)). Fragments of ICAM-1 from which the transmembrane domain has been deleted, and which possess at least domains 1 and 2, are soluble under physiological conditions and can block ICAM-1/receptor interactions (Becker *et al., J. Immunol 147*:4398-4401 (1991)).

Soluble derivatives of CD18 family members comprise another type of the Group I agents of the present invention. As used herein, a molecule is a member of the CD18 family of glycoproteins if it contains either an alpha subunit of a member of the CD18 family of glycoproteins (i.e., a CD11 subunit), a beta subunit of a member of the CD18 family of glycoproteins (i.e., a CD18 beta subunit), or both an alpha and a beta subunit of a member of the CD18 family of glycoproteins. Thus, as used herein, a member of the CD18 family of glycoproteins includes molecules having only one subunit of a CD18 member as well as heterodimers, (molecules having both an alpha and a beta subunit of a member of the CD18 family). Soluble derivatives of members of the CD18 family have been generated by deleting the transmembrane domain (Dana *et al., Proc. Natl. Acad. Sci. USA 88*:3106-3110 (1991)). These molecules have been shown to reduce the rate of ICAM-1/ligand binding by binding to ICAM-1.

There are numerous procedures known in the art to assay for ICAM-1/receptor interactions. These can be used by a skilled artisan, without undue experimentation, to identify and isolate additional Group I and Group II agents for use in the herein disclosed methods. The agents screened in such assays can be, but are not limited to, peptides, carbohydrates, small molecules, or vitamin derivatives. The agents can be selected and screened at random or rationally selected or designed using known protein modeling techniques. For random screening, agents such as peptides or carbohydrates are selected at random and are assayed for the ability to bind to ICAM-1 or a CD18 family member. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the

agent is chosen based on the molecular configuration of the ICAM-1 or a CD18 family member. For example, one skilled in the art can readily adapt currently available procedures to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides (for example, see Hurby *et al.*, "Application of Synthetic Peptides: Antisense Peptides", In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY, pp. 289-307 (1992), and Kaspczak *et al., Biochemistry* 28:9230-8 (1989)).

The agents of the present invention can be used in native form or can be modified to form a chemical derivative. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980)).

For example, a change in the immunological character of the functional derivative, such as affinity for a given antibody, is measured by a competitive type immunoassay. Changes in immunomodulation activity are measured by the appropriate assay. Modifications of such protein properties as redox or thermal stability, biological half-life, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

The therapeutic effects of the agents of the present invention may be obtained by providing the agent to a patient by any suitable means (i.e., inhalation, intravenously, intramuscularly, subcutaneously, enterally, or parenterally). It is preferred to administer the agent of the present invention so as to achieve an effective concentration within the blood or within the lungs. For achieving an effective concentration within the lungs, the preferred method is to administer the agent as a nebulized solution by oral inhalation,

or via an oral spray or oral aerosol. Alternatively, intra-nasal or intratracheal administration can be employed to achieve an effective lung concentration.

To achieve an effective blood concentration, the preferred method is to administer the agent by injection. The administration may be by continuous infusion, or by single or multiple injections.

In providing a patient with antibodies, or fragments thereof, capable of binding to ICAM-1 or to a member of the CD18 family, or when providing a soluble form of ICAM-1 or a member of the CD18 family, the dosage of the administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc. In general, it is desirable to provide the recipient with a dosage of agent which is in the range of from about 1 pg/kg to 10 mg/kg (body weight of patient), although a lower or higher dosage may be administered. The therapeutically effective dose can be lowered by using combinations of the agents of the present invention (such as, for example, if anti-ICAM-1 antibody is additionally administered with an anti-LFA-1 antibody).

As used herein, two or more compounds are said to be administered "in combination" with each other when either (1) the physiological effects of each compound or (2) the serum concentrations of each compound can be measured at the same time. The composition of the present invention can be administered concurrently with, prior to, or following the administration of other anti-viral or anti-hyperresponsiveness agents.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to increase the rate of lung gas exchange and thus attenuate the morbidity (respiratory distress or dyspnea) of an infection of the respiratory tract.

The administration of the agent(s) of the invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the agent(s) are provided in advance of any decrease in the rate of gas exchange. The prophylactic administration of the agent(s) serves to prevent or attenuate any subsequent reduction in gas exchange. When provided

therapeutically, the agent(s) are provided at (or shortly after) the onset of a reduction in the rate of gas exchange. The therapeutic administration of the compound(s) serves to attenuate any actual reduction in gas exchange. Thus, the agents of the present invention may thus be provided after respiratory viral infection and either prior to the onset of a reduction in gas exchange (so as to attenuate the anticipated severity, duration or extent of the reduction) or after the initiation of the reduction.

The agents of the present invention are administered to the mammal in a pharmaceutically acceptable form and in a therapeutically effective concentration. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

The agents of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the agents of the present invention, together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb one or more of the agents of the present invention. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl,

pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another method to control the duration of action by controlled release preparations is to incorporate agents of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization yielding, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980)).

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Examples

Example 1

As mentioned above, respiratory viral infections, and in particular infection with respiratory syncytial virus (RSV), are a major cause of hospitalization in infants, the elderly and patients with cardiopulmonary restrictions. Several lines of evidence suggest that the morbidity of these infections is a consequence of the immune response rather than the cytopathic effects of the virus (Stott *et al., J. Virol. 61*:3855-3861 (1987); Murphy *et al., Vaccine 8*:497-502 (1990)). The objective of the experiments described in this

example was to characterize the time course of the immune/inflammatory response (leukocyte influx and cytokine generation) and viral replication induced by RSV, as well as the lung dysfunction and increase in airway responsiveness characteristic of asthma onset and severity (Busse *et al., J. Allergy Clin. Immunol. 81*:770-775 (1988)) at the peak of the inflammatory response in mice.

Female Balb/c mice, ~ 32 weeks of age, were inoculated by intranasal insufflation (inhalation) administration of 10^7 plaque forming units (PFU) of RSV A2 strain or control (HEp-2) media. The mice were sacrificed 1, 3, 6, 15 or 28 days later and (1) lung lavage was performed to assess leukocyte influx (Wegner *et al., Lung 170*:267-279 (1992)) and cytokine generation (EIA using commercially available kits), (2) lung homogenates were prepared and used in plaque assays on HEp-2 cells to assess viral replication (Graham *et al., J. Med. Virol 26*:153-162 (1988)), or (3) lungs were fixed for histologic examination. For evaluation of pulmonary function, the mice were anesthetized with pentobarbital and their tracheas cannulated with a 18 gauge catheter. Respiratory system resistance (Rrs) and dynamic compliance (Crs) were determined from respiratory system impedance which was measured by discrete frequency (4 to 40 Hz in 11 equal logarithmic steps) sinusoidal-forced oscillations superimposed on tidal breathing (Wegner *et al., Lung 170*:267-279 (1992)). Diffusion capacity of the lungs for carbon monoxide (D_{LCO}) was determined by the single breath method. Airway responsiveness was assessed by determining the concentration of nebulized and inhaled methacholine to produce a 100% increase in Rrs (PC_{100}). This was accomplished by administering increasing concentrations of methacholine (diluted with phosphate-buffered saline) in half-logarithmic steps (at ~ 10 minute intervals) until a > 100 % increase in Rrs from baseline was obtained. The PC_{100} was then calculated by linear regression analysis of the last 2 or 3 points on the logarithmic methacholine concentration versus percent increase in Rrs plot.

Results:

Viral titers peaked at day 3, leukocyte influx at day 6, TNFα, GMCSF and IL-6 generation at day 1, and IFN-γ at day 6 (see Table 2). On day 6 (peak inflammation), RSV infection decreased lung diffusion capacity (D_{LCO}: 16.6 ± 0.5 to 14.0 ± 0.6 μl/min/mmHg, $p < 0.05$) and increased airway responsiveness (decreased the methacholine PC_{100}: 0.72 ± 0.22 to 0.06 ± 0.02 mg/ml, $p < 0.05$) without significantly altering Rrs (797 ± 87 to 799 ± 71 $cmH_2O/l/s$) or Crs (35.8 ± 5.7 to 28.3 ± 2.8 ul/cmH_2O).

Thus, RSV infection in mice induces a marked lung inflammation and dysfunction.

Table 2

Time Course of RSV-Induced Viral Replication, Leukocyte Influx and Cytokine Generation in Mice

Day	Body Weight (% Change)	Viral Titer	Lavage Total Cells (x10^5)	Lavage Cytokines (pg/ml)			
				TNFα	GMCSF	IL-6	IFN-γ
0	0	0	3.9±0.5	BDL	BDL	BDL	BDL
1	-1.7±0.4	0 - 1^+	8.4±1.2	696±135*	104±19*	1657±112*	0.4±0.1
3	-1.8±0.6	3^+ - 4^+	10.9±0.8*	BDL	BDL	455±166*	0.2±0.1
6	-5.7±1.5*	2^+ - 3^+	15.4±1.3*	BDL	BDL	443±60*	19.2±2.3*
15	-4.8±0.7*	0	12.6±1.1*	BDL	BDL	BDL	BDL
28	-3.2±1.2*	0	6.5±1.3*	---	---	---	---

BDL = below detectable limits; * $p < 0.05$ vs. baseline and media inoculate controls by Student's t-test.

Example 2

Since RSV infection was found to stimulate the *in vivo* lung generation of cytokines (TNFα and IFNγ) known to increase ICAM-1 expression *in vitro,* immunohistochemistry was employed to determine if inoculation (infection) with RSV enhanced ICAM-1 expression *in vivo.*

Female Balb/c mice, ~ 32 weeks of age, were inoculated by intranasal insufflation (inhalation) administration of 10^7 plaque forming units (PFU) of RSV A2 strain or control (HEp-2) media and then sacrificed 6 days later. Lungs were removed, inflated with O.C.T. and then frozen in liquid nitrogen. After being cryosectioned, 5-10 μm sections were fixed in acetone, stained with the rat anti-mouse ICAM-1 monoclonal antibody YN1/1.7 or rat IgG (as a control for non-specific binding), and developed using biotinylated goat anti-rat IgG linked with peroxidase-conjugated streptavidin and visualized with 3-amino-9-ethylcarbazole (AEC) (Wegner *et al., Lung 170*:267-279 (1992)). The sections were counterstained with Mayer's hematoxylin.

Results:

As reported previously (Wegner *et al., Lung 170*:267-279 (1992); Kang *et al., Am. J. Respir. Cell Molecular Biol. 9*:350-355 (1993)), a slight but distinct basal level of ICAM-1 expression was observed on the peripheral lung parenchymal cells (mostly on type 1 pneumocytes) and alveolar macrophages in naive (normal) mice as well as in those inoculated with control media. Infection with RSV induced a marked enhancement of ICAM-1 expression on lung parenchymal cells as well as on alveolar macrophages and infiltrating mononuclear leukocytes. Little, if any, non-specific background staining was observed even in the RSV infected mice.

Thus, ICAM-1 expression is strikingly upregulated on lung cells and infiltrating leukocytes after RSV infection.

Example 3

The contribution of ICAM-1 to the inflammatory response, attenuated alveolar gas exchange and increase in airway responsiveness induced by RSV infection was evaluated using the rat anti-mouse ICAM-1 monoclonal antibody YN1/1.7.

Female Balb/c mice were inoculated by intranasal insufflation (inhalation) administration of 10^7 plaque forming units (PFU) of RSV A2 strain or control (HEp-2) media and treated twice daily (b.i.d.) beginning 1 hr prior to RSV inoculation with YN1/1.7 or rat IgG at 3 mg/kg, intraperitoneal. On day six after inoculation (peak inflammation), lung lavage leukocyte counts (inflammation), D_{LCO} (lung gas exchange), and the inhaled methacholine PC_{100} (airway responsiveness) were determined as described above in Example 1.

Results:

RSV infection induced a marked influx of mononuclear cells that was significantly inhibited (65 - 80%) by YN1/1.7 (anti-ICAM-1) but not by control rat IgG (Figure 1). Likewise, the RSV-induced decrease in lung gas exchange (D_{LCO}) was significantly and completely attenuated by YN1/1.7 treatment but not by control rat IgG (Figure 2). In contrast, the RSV-induced increase in airway responsiveness (decrease in methacholine PC_{100}) was not inhibited by YN1/1.7 treatment (Figure 3).

These results indicate that the acute symptoms of dyspnea associated with impaired alveolar gas exchange are linked to the intense inflammatory response (leukocyte infiltration) induced by RSV infection and can be impressively attenuated by blocking ICAM-1/receptor interactions. However, the more chronic asthma-like wheezing symptoms, which are likely due to an RSV-induced increase in airway responsiveness, are not the result of the inflammatory response but rather possibly the cytopathic effects of the virus.

Thus, the impaired lung gas exchange that causes the acute morbidity and hospitalization associated with respiratory viral infections can be impressively attenuated by antagonism of ICAM-1/receptor interactions. However, optimal therapy which includes the prevention of the onset of asthma would require combination of this ICAM-1 antagonism with the concomitant administration of an anti-viral agent (e.g. Ribavirin for RSV).

What Is Claimed Is:

1. A method for increasing the rate of gas exchange in the lungs of a mammal suffering from reduced lung gas exchange as a result of viral infection of the respiratory tract which comprises providing to said mammal a therapeutically effective amount of an agent selected from the group consisting of:

(a) an antibody which binds to ICAM-1;

(b) a fragment of said antibody (a), said fragment containing the antigen binding site of said antibody;

(c) ICAM-1, substantially free of natural contaminants;

(d) a soluble derivative of ICAM-1;

(e) an antibody which binds to a member of the CD18 family of glycoproteins;

(f) a fragment of said antibody (e), said fragment containing the antigen binding site of said antibody;

(g) a member of the CD18 family of glycoproteins, substantially free of natural contaminants; and

(h) a soluble derivative of a member of the CD18 family of glycoproteins;

wherein said viral infection results in an induction of the expression of ICAM-1.

2. The use of an agent which increases the rate of gas exchange in the lungs of a mammal for the manufacture of a therapeutic composition for the treatment of a viral infection of the respiratory tract of said mammal, wherein said viral infection results in an induction of the expression of ICAM-1, and wherein said agent is selected from the group consisting of:

(a) an antibody which binds to ICAM-1;

(b) a fragment of said antibody (a), said fragment containing the antigen binding site of said antibody;

(c) ICAM-1, substantially free of natural contaminants;

(d) a soluble derivative of ICAM-1;

(e) an antibody which binds to a member of the CD18 family of glycoproteins;

(f) a fragment of said antibody (e), said fragment containing the antigen binding site of said antibody;

(g) a member of the CD18 family of glycoproteins, substantially free of natural contaminants; and

(h) a soluble derivative of a member of the CD18 family of glycoproteins;

wherein said viral infection results in an induction of the expression of ICAM-1.

3. The method of claim 1 or the use of claim 2, wherein said agent is said antibody (a) or said fragment (b) of said antibody (a).

4. The method or use of claim 3, wherein said antibody (a) is a monoclonal antibody.

5. The method or use of claim 4, wherein said monoclonal antibody is the monoclonal antibody R6.5.

6. The method of claim 1 or the use of claim 2, wherein said agent is said ICAM-1 (c).

7. The method of claim 1 or the use of claim 2, wherein said agent is said soluble derivative of ICAM-1(d).

8. The method or use of claim 7, wherein said soluble derivative of ICAM-1 contains ICAM-1 domain 1.

9. The method or use of claim 7, wherein said soluble derivative of ICAM-1 contains ICAM-1 domain 3.

10. The method of claim 1 or the use of claim 2, wherein said agent is said antibody (e) or said fragment (f).

11. The method or use of claim 10, wherein said antibody (e) binds to an alpha subunit of the CD18 family of glycoproteins.

12. The method or use of claim 10, wherein said antibody (e) binds to a beta subunit of the CD18 family of glycoproteins.

13. The method of claim 1 or use of claim 2, wherein said agent is a member of the CD18 family of glycoproteins, substantially free of natural contaminants.

14. The method or use of claim 13, wherein said member of the CD18 family of glycoproteins contains an alpha subunit of a member of the CD18 family of glycoproteins.

15. The method or use of claim 13, wherein said member of the CD18 family of glycoproteins contains a beta subunit of a member of the CD18 family of glycoproteins.

16. The method or use of claim 14, wherein said member of the CD18 family of glycoproteins is a heterodimer containing both an alpha and a beta subunit of a member of the CD18 family of glycoproteins.

17. The method of claim 1 or use of claim 2, wherein said viral infection is caused by a member of the Paramyxoviradae family.

18. The method or use of claim 17, wherein said member of the Paramyxoviradae family is a member of the Pneumovirus genus.

19. The method or use of claim 18, wherein said member of the Pneumovirus genus is a Respiratory Syncytial Virus.

20. The method or use of claim 17, wherein said member of the Paramyxoviradae family is a member of the Paramyxovirus genus.

21. The method or use of claim 20, wherein said member of the Paramyxovirus genus is a parainfluenza virus.

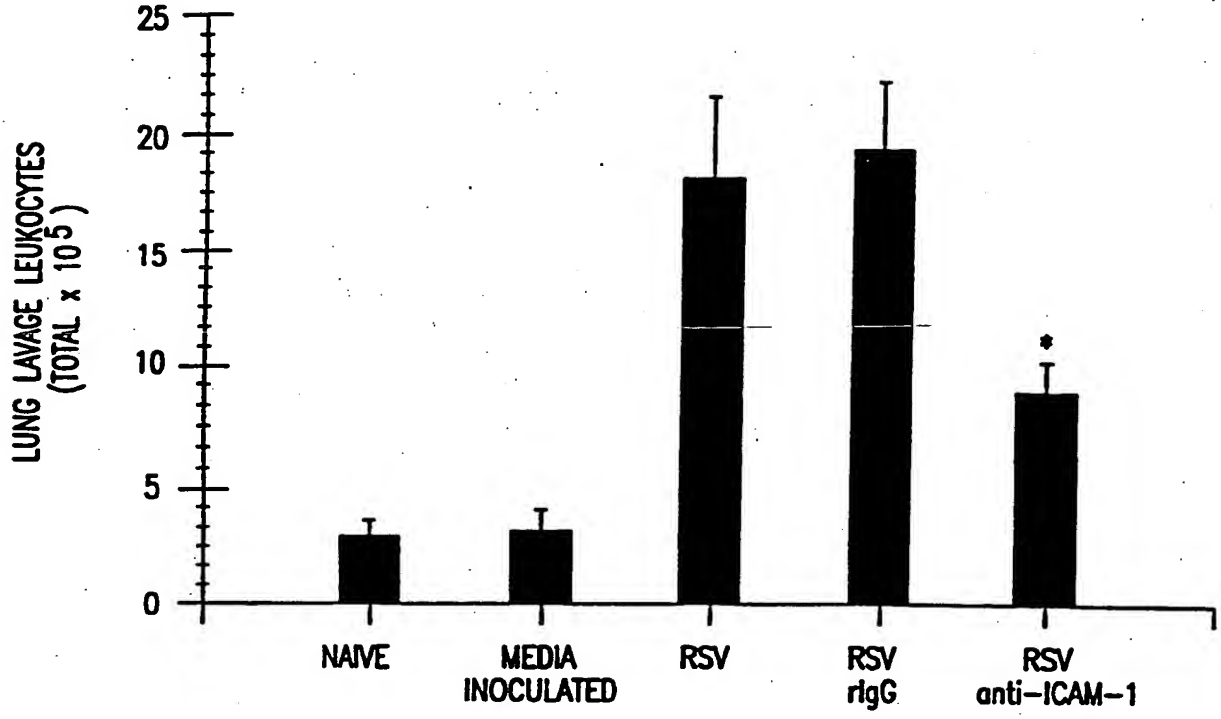
LUNG LAVAGE LEUKOCYTES
(TOTAL x 10^5)
25
20
15
10
5
0
*
NAIVE
MEDIA INOCULATED
RSV
RSV rIgG
RSV anti-ICAM-1

FIG.1

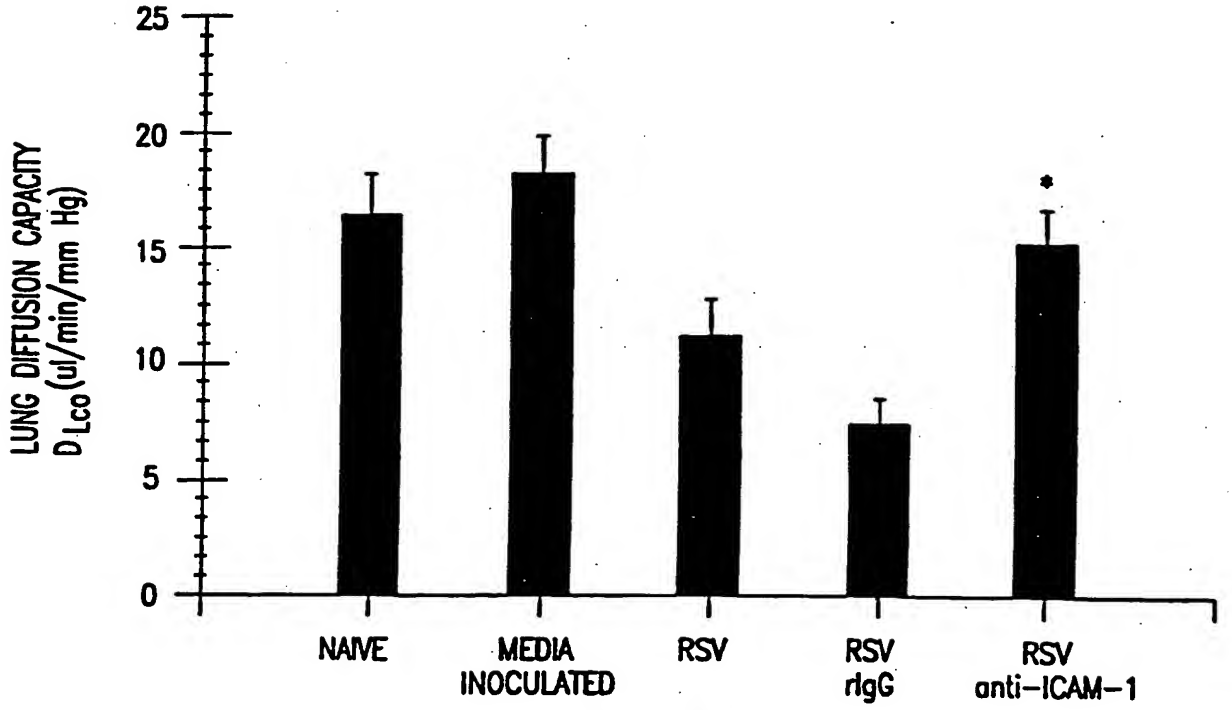
LUNG DIFFUSION CAPACITY
D_{Lco} (ul/min/mm Hg)
25
20
15
10
5
0
*
NAIVE
MEDIA INOCULATED
RSV
RSV rIgG
RSV anti-ICAM-1

FIG.2

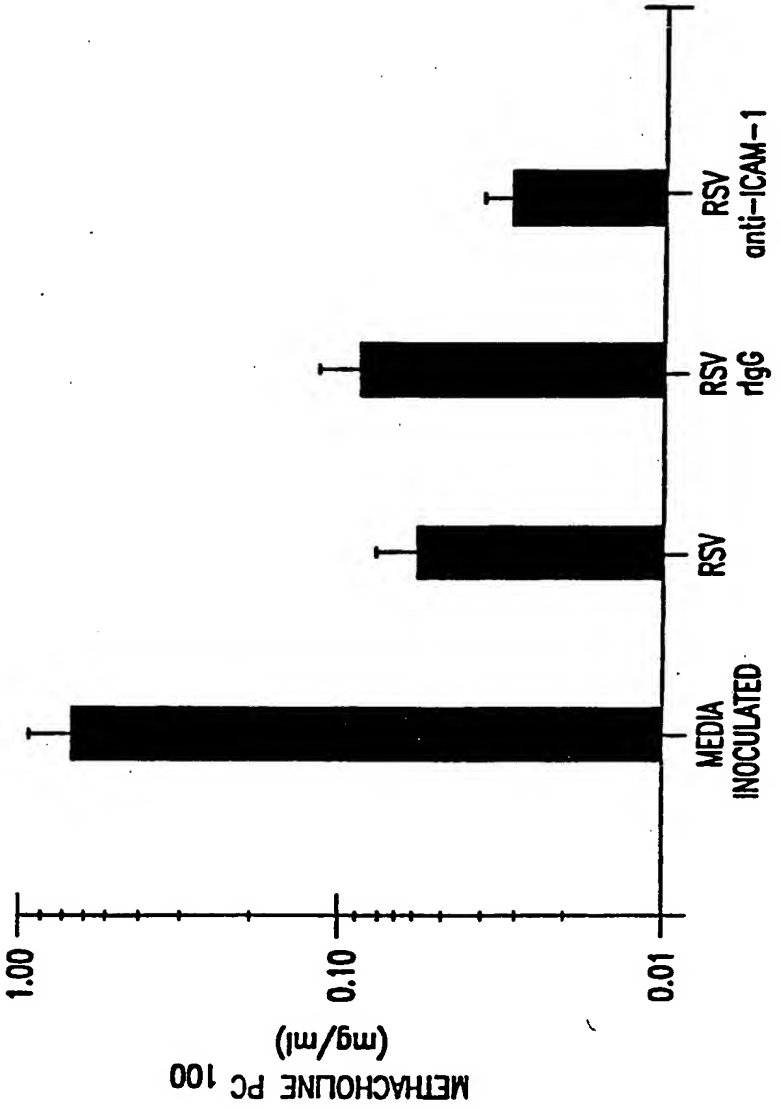
METHACHOLINE PC 100 (mg/ml)
1.00
0.10
0.01
MEDIA INOCULATED
RSV
RSV IgG
RSV anti-ICAM-1

FIG.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 16/28; A61K 39/265, 39/42, 39/00
US CL :424/130.1, 141.1, 143.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 141.1, 143.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemistry, Vol. 90, issued January 1993, N.H. Olson et al., "Structure of a human rhinovirus complexed with its receptor molecule", pages 507-511, see entire document.	1-5, 17-21
Y	Proc. Natl. Acad. Sci., Vol. 88, issued September 1991, A. McClelland et al., "Identification of monoclonal antibody epitopes and critical residues for rhinovirus binding in domain 1 of intercellular adhesion molecule 1", pages 7993-7997, see entire document.	1-5, 17-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 JULY 1995	Date of mailing of the international search report 31 JUL 1995
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Virol., Vol. 65, No. 11, issued November 1991, J.M. Greve et al., "Mechanisms of receptor-mediated rhinovirus neutralization defined by two soluble forms of ICAM-1", pages 6015-6023, see entire document.	1-5, 17-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 17-21

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows: anti-ICAM-1 antibody, ICAM-1, anti-CD18, and CD18.

The following claims are generic: 1, 2, 17-21.

The claims are deemed to correspond to the species listed above in the following manner:

Species I, claim(s) 3, 4, 5, drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being anti-ICAM-1 antibody.

Species II, claim(s) 6-9, drawn to drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being ICAM-1.

Species III, claim(s) 10-12, drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being anti-CD18 antibody.

Species IV, claim(s) 13-16, drawn to drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being CD18.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

The ICAM-1 and CD18 are membrane-bound proteins on differing cells which bind to each other. ICAM-1 and CD18 are each comprised of differing structure and amino acid composition and the CD18 can be used to bind to other proteins such as ICAM-2. ICAM-1 does not bind ICAM-2. The special technical features that identify ICAM-1 and CD18 distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

The anti-ICAM-1 antibody and ICAM-1 differ in composition and function. ICAM-1 can be used to stimulate the immune system or to isolate CD18 while the anti-ICAM-1 antibody can be used to isolate ICAM-1, inhibit its actions, or increase its half-life. The special technical features that identify anti-ICAM-1antibodyand ICAM-1 distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

The anti-CD18 antibody and CD18 differ in compositionandfunction. CD18 binds ICAM-1 while the anti-CD18 antibody binds CD18 and not ICAM-1 The special technical features that identify anti-CD18 antibody and CD18 distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

The anti-ICAM-1 and anti-CD18 antibodies are distinct because these antibodies bind to different proteins that are distinct for the reasons identified above. The special technical features that identify anti-ICAM-1 and anti-CD18 antibodies distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

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(54) Title: USE OF AGENTS WHICH BLOCK INTERCELLULAR ADHESION MOLECULE/RECEPTOR INTERACTION IN THE TREATMENT OF RESPIRATORY VIRAL INFECTION

(57) Abstract

The present invention relates to the use of intercellular adhesion molecules (ICAM-1), their functional derivatives, and molecules which bind to them, in methods to increase gas exchange in the lungs of a patient suffering from a respiratory viral infection.

* (Referred to in PCT Gazette No. 12/1996, Section II)

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GA	Gabon				

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 16/28, 14/705; A61K 39/265, 39/42, 39/00
US CL :424/130.1, 141.1, 143.1; 530/350, 395
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 141.1, 143.1; 530/350, 395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Biochemistry, Volume 90, issued January 1993, N.H. Olson et al., "Structure of a human rhinovirus complexed with its receptor molecule", pages 507-511, see entire document.	1-21
A	Proc. Natl. Acad. Sci., Volume 88, issued September 1991, A. McClelland et al., "Identification of monoclonal antibody epitopes and critical residues for rhinovirus binding in domain 1 of intercellular adhesion molecule 1", pages 7993-7997, see entire document.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
11 JANUARY 1996	08 FEB 1996

Name and mailing address of the ISA/US	Authorized officer
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. Virol., Volume 65, Number 11, issued November 1991, J.M. Greve et al., "Mechanisms of receptor-mediated rhinovirus neutralization defined by two soluble forms of ICAM-1", pages 6015-6023, see entire document.	1-21
A	Immunological Reviews, Number 108, issued 1989, S.O. Wawryk, et al., "The role of the LFA-1/ICAM-1 interaction in human leukocyte homing and adhesion", pages 135-161, see entire document.	1-21
A	J. Virology, Volume 64, No. 6, issued June 1990, D.W. Lineberger et al., " Antibodies that block rhinovirus attachment map to domain 1 of the major group receptor", pages 2582-2587, see entire document.	1-21
A, P	Antimicrobial Agents and Chemotherapeutics, Volume 38, No. 6, issued June 1994, C.E. Crump et al., "Comparative antirhinoviral activities of soluble intercellular adhesion molecule-1 (sICAM-1) and chimeric ICAM-1/immunoglobulin A molecule", pages 1425-1427, see entire document.	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ **Claims Nos.:**
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ **Claims Nos.:**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ **Claims Nos.:**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04519

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows: anti-ICAM-1 antibody, ICAM-1, anti-CD18, and CD18.

The following claims are generic: 1, 2, 17-21.

The claims are deemed to correspond to the species listed above in the following manner:

Species I, claims 3, 4, 5, drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being anti-ICAM-1 antibody.

Species II, claims 6-9, drawn to drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being ICAM-1.

Species III, claims 10-12, drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being anti-CD18 antibody.

Species IV, claims 13-16, drawn to drawn to a method or use of an agent which increases the rate of gas exchange in the lungs of a mammal for the treatment of a viral infection, the agent being CD18.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

The ICAM-1 and CD18 are membrane-bound proteins on differing cells which bind to each other. ICAM-1 and CD18 are each comprised of differing structure and amino acid composition and the CD18 can be used to bind to other proteins such as ICAM-2 whereas ICAM-1 does not bind ICAM-2. The special technical features that identify ICAM-1 and CD18 distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

The anti-ICAM-1 antibody and ICAM-1 differ in composition and function. ICAM-1 can be used to stimulate the immune system or to isolate CD18 while the anti-ICAM-1 antibody can be used to isolate ICAM-1, inhibit its actions, or increase its half-life. The special technical features that identify anti-ICAM-1 antibody and ICAM-1 distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

The anti-CD18 antibody and CD18 differ in composition and function. CD18 binds ICAM-1 while the anti-CD18 antibody binds CD18 and not ICAM-1 The special technical features that identify anti-CD18 antibody and CD18 distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

The anti-ICAM-1 and anti-CD18 antibodies are distinct because these antibodies bind to different proteins that are distinct for the reasons identified above. The special technical features that identify anti-ICAM-1 and anti-CD18 antibodies distinguish one from the other. The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

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